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Attempts to establish New Experimental Methods to study Antitumoral Drugs *

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The possibility to detect new drugs for any type of therapy is frequently associated to the availability of new experimental methods. This paper intends to summarize some of the recent attempts made in this laboratory along this line. Three main attempts will be discussed: transplantation of tumours in different sites of the body and their sensitivity to cancer chemotherapy; a method to study cancer dissemination and effects of various drugs on this system; perfusion of tissue cultures with an *in vivo-in vitro* system.

Chemotherapy of tumours transplanted into the brain

The method used to implant cancer cells into the brain of rats and mice has been previously described in detail (Rosso *et al.*, 1966*a*). Tumours found most suitable for this study were an uterine epithelioma (T_8 of Guerin) for rats and Sarcoma 180 and Ehrlich carcinoma maintained in the ascite form for mice.

Extensive studies performed particularly in mice showed that as low as 100 cells are able to produce tumour growth in almost the totality of the mice injected intracerebrally. There was a progressive decrease of the survival time (Rosso *et al.*, 1966; Rosso and Palma, 1966) by increasing the number of cells from 100 to 1 million. In this test system the effect of several drugs was tested. 1 methyl-nitroso urea (MNU) was able to increase the survival time of mice implanted intracerebrally with Sarcoma-180.

The efficacy was proportional to the dose used and inversally proportional to the number of cells injected. A prolongation of the survival time was achieved not only when the treatment started before the transplantation but also when MNU was given at about half the expected survival time of mice bearing an intracerebral tumour.

In contrast with this reproducible effect MNU didn't show appreciable action on Sarcoma 180 transplanted subcutaneously (5 million cells) or intraperitoneally

* This work was supported by Euratom (Contract 040-65-1-BIOI) and by Consiglio Nazionale delle Ricerche (Contract 115/1134/1000).

(1 million cells). Similar results were obtained when Ehrlich carcinoma or an uterine epithelioma were used (Rosso and Palma, 1966; Rosso *et al.*, 1966*b*).

Among several congeners of MNU only the bischloroethyl amino derivative (BCNU) was found effective while urea, hydroxyurea, ethylcarbamate, methylnitrosonitroguanidine and butylnitrosoguanidine were pratically inactive.

Known chemotherapeutical agents including DL-Sarcolysine, cyclophosphamide, vinblastine, vincristine, actinomycin D and methotrexate were not active in prolonging the survival time of animals implanted intracerebrally with tumors even when they were effective on the same tumour transplanted subcutaneously and intraperitoneally.

Tab. 1 summarizes in a schematic form some of the results obtained.

Tab. 1. Effect of different antitumoral drugs on sarcoma 180 growing in different sites

Drug	Dose mg/kg i.p. × 6 days	N. of sarcoma 180 cells injected				
		I.C.			S.C.	I.P.
		1 000 000	100 000	1 000	5 000 000	1 000 000
MNU	16	+	+++	+++	—	—
MNU	8	++	+++	++	—	—
MNU	4	++	+++	—	—	—
DL-Sarcolysine	4.2		—		+	+++
DL-Sarcolysine	2.1		—		—	+++
Cyclophosphamide	85		—		+++	+++

I.C. = Intracerebrally
S.C. = Subcutaneously
I.P. = Intraperitoneally

The fact that a differential efficacy in the chemotherapeutic treatment was achieved with the same drug and with the same tumour transplanted in different sites suggests new possibilities of research.

Furthermore MNU appears enough active to warrant possible clinical trials and to suggest new leads in terms of structure activity relationship.

Cancer dissemination

The malignancy of tumours is mostly the result of the metastatic dissemination of cancer cells. This problem is difficult to study experimentally because most animal tumours used for cancer research do not induce reproducible and measurable metastases. Several authors in the effort to find a model for this type of investigation use to inject cancer cells intravenously (Fisher and Fisher, 1959; Clifton, 1966; D'Agostino, 1964).

This method, although having the advantage to be quantitatively controlled, is rather remote from the clinical situation because: *a*) there is not a primary tumour able to supply cancer cells continuously; *b*) usually a great number of cells must be injected; *c*) normal animals are used instead of animals with depressed defences because of the tumour growth.

In order to carry out experimental models suitable for an approach to the problem of cancer cell dissemination into the blood and peripheral tissues from a primary tumour, rats or mice with a tumour transplanted into the brain have been used.

Studies concerning the dissemination of an uterine epithelioma transplanted intracerebrally in rats has been already published (Rosso *et al.*, 1966*b*).

It was shown that this tumour disseminates in a short time in blood and lung but only occasionally in liver, kidney and lymphonodes.

The dissemination was much more pronounced and reproducible when the tumour was transplanted into the brain than in the usual localization, namely subcutaneously or intraperitoneally.

Similar studies have been carried out also in mice by implanting into the brain Ehrlich carcinoma or Sarcoma 180. The method to detect cancer dissemination was an indirect one. It consisted in transplanting, at given times, blood clot or fragments of tissues obtained from mice bearing an intracerebral tumour, subcutaneously in normal recipient mice.

Tab. 2 reports the data obtained in these experiments. It is quite evident that

Tab. 2. Dissemination of cancer cells from tumours growing subcutaneously or intracerebrally

	Ehrlich carcinoma		Sarcoma 180	
	S.C.*	I.C.**	S.C.*	I.C.**
Survival time (days)		12.0 ± .4		12.4 ± .3
Growth of primary tumour (at 14 days/mg)	827 ± 110		1820 ± 205	
Total number of takes in recipient mice for:				
blood	22	35	14	28
lung	16	30	4	23
liver	8	4	3	7
kidney	13	1	2	1
axillary limphonodes	18	—	7	n.d.
Total	77	70	30	59
Maximal possible	280	160	280	160
%	27.5	43.7	10.7	36.8

* The subcutaneous tumour was obtained injecting 5 000 000 tumour cells/0.2 ml/mouse/s.c.
** The intracerebral tumour was obtained injecting 500 000 tumour cells/0.01 ml/mouse/i.c.

cancer dissemination was very pronounced when the tumour was transplanted intracerebrally in respect to the subcutaneous localization.

Using this system, it was possible to show that hydrocortisone was able to increase the presence of cancer cells in the lungs of animals bearing an intracerebral tumour, as it is evident from Tab. 3.

Among cytotoxic drugs MNU and cyclophosphamide were able to decrease cancer cells dissemination in blood and lung. The effect was evident only during the treatment. The significance of these results remains to be demonstrated for the possibility that the cytotoxic drugs taken up by the cancer cells was continuing its effect when blood clot or fragments of lung were transplanted subcutaneously.

Tab. 3. Effect of hydrocortisone (50 mg/kg/i.p. daily) on cancer cells dissemination in mice bearing intracerebrally sarcoma 180 (100 000 cells/mouse)

Days after I.C. transplantation of sarcoma 180	Percentages of takes for					
	Brain		Blood		Lung	
	Controls	Hydrocortisone	Controls	Hydrocortisone	Controls	Hydrocortisone
3	87	75	37	37	12	100
7	100	100	50	62	50	100
10	100	100	75	87	87	100

Hydrocortisone treatment started the day after the intracerebral transplantation.

Perfusion of tissue cultures

The very low chemotherapeutic index of antitumoral drugs requires careful control of doses and schedules of treatment for a given drug. It is obvious that the knowledge of the blood concentration of an antitumoral drug may be extremely useful to solve this problem.

The chemical determination is not always possible for the low concentration of the drug used and the difficulty to know if a given drug is active per se or through the formation of a metabolite.

It was therefore interesting to set up a method with the aim to study in a dynamic way the cytotoxicity of the blood for in vitro tissue culture. The interest for this research was reinforced by the frequent observation that drugs extremely active in tissue culture are sometimes almost inactive on tumour transplanted *in vivo*.

The experimental condition used in this laboratory has been previously reported in details (Morasca and Rainisio, 1966*a*). In practice, silicone tubing are inserted in the proximal and distal part of the rat femoral artery. Between the two tubing is placed a modified Rose chamber where in one compartment the blood is circulating and in the other compartment the tissue culture is growing.

The two compartments are separated by a membrane with pores of the diameter of 20-35 mμ. The circulation of the blood doesn't require a pump.

The animal is pretreated 48 hours before beginning of experiments with 8 mg/kg of Warfarin. Two hours before beginning of experiments, the animal was spinalized by injecting 0.05 ml of ethanol in the lumbar spinal cord. In this system it is therefore possible to inject drugs into the animal and to follow the presence of cytotoxic effects in the tissue culture.

Fig. 1 reports an example of the usefulness of this method. Three alkaloids of Catharantus Roseus have been studied, namely Vinblastine, Vincristine and Vinleurosine. It is evident that cytotoxic effect of the blood is longer lasting in the case of Vinblastine than for Vincristine or Vinleurosine. It is interesting to recall that in vitro Vincristine results several times more active than Vinblastine (Morasca and Rainisio, 1966*b*).

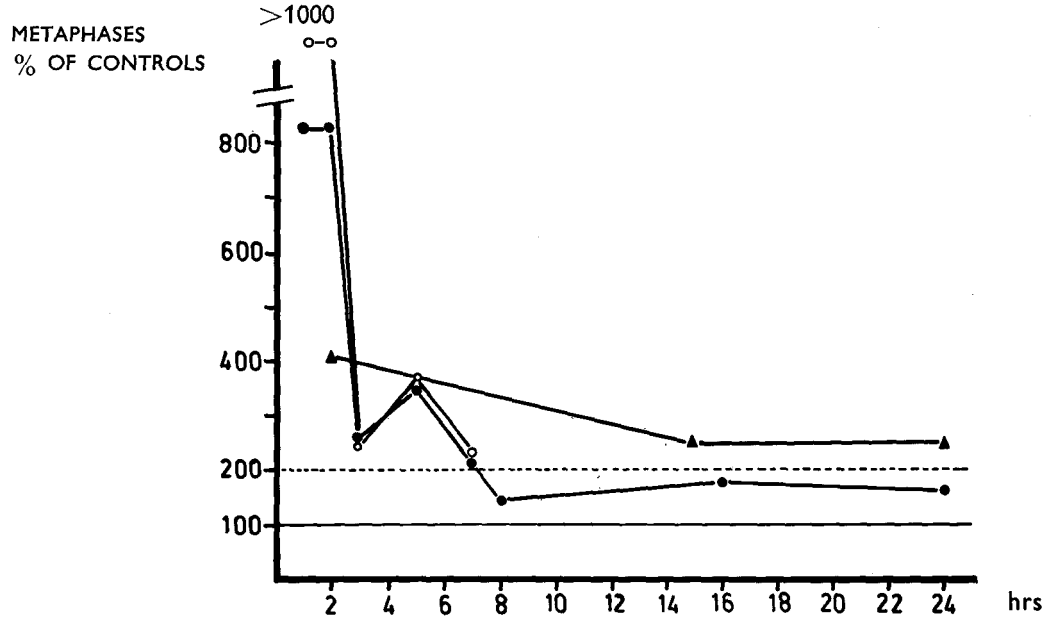

Fig. 1. Effect of Vinca Alkaloids on perfused tissue culture. Vinblastine (VLB) ▲- Vincristine (VCR) o- and Vinleurosine (VLR) ●- have been given at different times before the perfusion. On the ordinates are reported the metaphases (as % of controls); on the abscissae the time of perfusion in hrs

The method used may permit to establish for each drug the optimal condition in which a sustained cytotoxic effect is achieved.

This information may in turn permit to assess the best schedule of treatment for tumours transplanted *in vivo*.

Summary

This paper summarizes some studies recently carried out in this Institute in the field of cancer chemotherapy.

Three main groups of data are reported:

a) different sensitivity to chemotherapy of the same tumor transplanted in different sites of the body;

b) a method to study cancer dissemination as a basis for cancer chemotherapy;

c) a system to perfuse tissue culture with the blood of living animals as an approach to study duration of action of antitumoral drugs.

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RIASSUNTO

Mediante la descrizione di alcune nuove esperienze nel campo della terapia del cancro, vengono discussi:

a) la diversa sensibilità alla chemioterapia dello stesso tumore trapiantato in sedi diverse;

b) un metodo per lo studio della disseminazione del cancro come base per la chemioterapia;

c) un sistema di perfusione di una cultura di tessuti col sangue di animali viventi soprattutto per studiare la durata dell'attività di alcuni medicamenti antitumorali.

RÉSUMÉ

On décrit quelques nouvelles expériences dans le domaine de la thérapie du cancer.

Trois points principaux sont discutés:

a) la différente sensibilité à la chimiothérapie de la même tumeur transplantée dans différents sièges;

b) une méthode pour étudier la dissémination du cancer comme base pour la chimiothérapie;

c) un système de perfusion d'une culture de tissu avec du sang d'animaux vivants surtout pour étudier la durée d'activité de quelques médicaments antitumoraux.

ZUSAMMENFASSUNG

Man beschreibt einige der letzten Versuche, die in unserem Institut durchgeführt worden sind auf dem Gebiet der Chemotherapie des Krebses.

Man bespricht drei Hauptgruppen von Versuchen:

a) die verschiedene Sensibilität zur Chemotherapie desselben Tumors implantiert in verschiedenen Körperstellen;

b) eine Methode um die Krebs-Dissemination zu studieren als Grund für die Chemotherapie des Krebses;

c) ein Perfusionssystem von Gewebe-Züchtungen mit Blut lebendiger Tiere hauptsächlich im Hinblick auf die Wirkungsdauer einiger antitumoraler Verbindungen.

https://doi.org/10.1017/S1120962300026299 Published online by Cambridge University Press